University of Zurich[UZH]

Master Thesis

# Multiple independent origins of reversed sexual size dimorphism:

## The role of sexual selection for male size in the black scavenger fly *Sepsis neocynipsea* (Diptera: Sepsidae)

Patrick T. Rohner

July 2015

Published as Rohner, P.T., Blanckenhorn, W.U. & N. Puniamoorthy. 2016. Sexual selection on male size drives the evolution of male-biased sexual size dimorphism via the prolongation of male development. **Evolution 70, 1189-1199.** doi:10.1111/evo.12944

Supervision by:
Prof. Dr. Wolf U. Blanckenhorn
Dr. Nalini Puniamoorthy

University of Zurich
Institute of Evolutionary Biology and Environmental Studies

# Contents

# Multiple origins of reversed sexual size dimorphism:

## The role of sexual selection for male size in the black scavenger fly *Sepsis neocynipsea* (Diptera: Sepsidae)


## Abstract

Sexual size dimorphism (SSD) arises when the net effects of natural and sexual selection for body size equilibrate differently between males and females. Species and even populations within a species can exhibit quantitative variation in the extent of SSD but directional reversals in SSD are rare in the animal kingdom. A previous study documented that sexual selection accounts for a geographic reversal of SSD in the widespread sepsid fly *Sepsis punctum* (Diptera: Sepsidae). Here, I study the variation in sexual selection acting on male body size in another closely related species that displays contrasting reversals of SSD in Europe (EU) and North America (NA). Using common garden experiments, I document that in *S. neocynipsea* females are larger in EU, whilst NA populations exhibit male-biased SSD. This reverses the situation of *S. punctum*, where NA populations display female-biased SSD while in EU males are larger. To verify the same underlying selection principle in both species, mating experiments were conducted under three increasingly male-biased operational sex ratios in *S. neocynipsea*. Selection differentials were calculated based on pairing success and clutch size to estimate the relative strength of sexual selection acting on male body size and fecundity selection acting on female size. In accordance with the differential equilibrium model of the evolution of SSD, the intensity of sexual selection on male size varies between continents in *S. neocynipsea* while no differences in fecundity selection were found. Additional comparative analyses of 25 sepsid taxa revealed four convergent origins of male-biased SSD. My results suggest rapid and independent evolution

of directional reversals in SSD in this group of flies, likely primarily mediated by sexual selection on male size, potentially in combination with mating system shifts, and proximately mediated more strongly by sexual divergence in growth rates rather than sexual bimaturism. Therefore, reversals in SSD and the associated variation in the ontogeny of body size might be much more rapid and common than currently assumed.

# Zusammenfassung

Sexualgrössendimorphismen (SGD) entstehen wenn sich die relativen Stärken von Sexueller und Natürlicher Selektion zwischen den Geschlechtern unterscheiden. Arten und Populationen innerhalb einer Art können sich in der Stärke der Ausprägung des SGD drastisch unterscheiden, Variation in der Richtung des SGD innerhalb einer Art ist im Tierreich jedoch äusserst selten. Eine frühere Studie belegt den Einfluss von Sexueller Selektion auf Männchengrösse in der Umkehrung des SGD zwischen Kontinentalpopulationen der weitverbreiteten Schwingfliegenart *Sepsis punctum* (Diptera: Sepsidae). In dieser Studie untersuche ich Selektionsdrücke auf Körpergrösse in einer anderen, nahverwandten Fliegenart, *Sepsis neocynipsea*, die ebenfalls eine Verkehrung des SGD zwischen Europa (EU) und Nordamerika (NA) zeigt. Mit Hilfe eines standardisierten Aufzuchtprotokolls demonstriere ich das in Europäischen Populationen Weibchen grösser als Männchen sind, wobei das Gegenteil auf Nordamerikanische Populationen zutrifft. Verpaarungsexperimente unter drei künstlichen Geschlechterverhältnissen wurden durchgeführt um die Stärke und Variation in der Sexuellen Selektion auf Männchengrösse zu messen. Selektionsdifferentiale wurden auf Grund von Paarungserfolg und Anzahl Nachkommen berechnet um den relativen Beitrag von Sexueller Selektion auf Männchengrösse und Fekunditätsselektion auf Weibchengrösse auf den Sexualgrössendimorphismus abzuschätzen. In Übereinstimmung mit dem Differentialgleichgewichts Modell der Evolution des SGD, variiert die Intensität der Sexuellen Selektion auf Männchengrösse zwischen den Kontinenten, wobei die Fekunditätsselektion auf Weibchengrösse konstant bleibt. Zusätzliche komparative Analysen basierend auf fünfundzwanzig Schwingfliegenarten deuten auf vier konvergente Ursprünge von SGD-Umkehrungen innerhalb dieser nahverwandten Arten hin. Meine Resultate legen eine schnelle und unabhängige Evolution von qualitativen Umkehrungen des SGD in der Gattung *Sepsis* nahe, die wahrschiendlich durch Sexuelle Selektion auf Männchengrösse verursacht werden und durch geschlechtsspezifische Wachstumsraten zustande kommen. Qualitative Umkehrungen des SGD könnten daher häufiger sein als momentan angenommen.

# Introduction

Sexual dimorphism, the divergence of morphological, behavioral and physiological traits between the sexes, is predicted to reflect the adaptive divergence between the sexes driven by variation in the optimal character states for each sex (Bateman, 1948; Hedrick & Temeles, 1989). Since the costs and benefits of mating virtually always differ between the sexes, sexual dimorphisms are ubiquitous in anisogamous species, encompassing sex-specific traits and differences in the mean phenotypic trait value of other shared characters between sexes (Bateman, 1948; Abouheif & Fairbairn, 1997; Fairbairn, 2013).

Due to its strong correlation with physiology and fitness (Peters, 1986; Reiss, 1991; Blanckenhorn, 2000), body size is especially subject to sexual dimorphism. Large females can generally allocate more energy to reproduction, providing more resources to more and/or higher quality offspring, thus fecundity selection usually favors larger body size in females (Darwin, 1872). In contrast, the strength and direction of selective pressures on male size, being caused by male-male competition and/or female choice, are more dependent on the mating system and the degree of male investment (Kokko *et al.*, 2014). Further, natural selection (eg. via predation or parasitism, ecological selection pressures, physiological constraints) is additionally shaping body size of both males and females. Following the differential equilibrium model of the evolution of SSD, the net effects of fecundity selection on female size, sexual selection on male size, and sex-specific natural (viability) selection shape the direction and strength of SSD (Price, 1984; Andersson, 1994; Blanckenhorn, 2000; Preziosi & Fairbairn, 2000; Fairbairn, 2013). Evolutionary changes of the mating system (eg. evolution of lekking behavior, high degrees of polyandry, etc.) or the environment (Teder & Tammaru, 2005) can thus result from and/or promote shifts in the direction and strength of SSD, as long as its evolvability is not hampered by the genetic architecture, strong genetic correlations between the sexes, or developmental constraints (Badyaev, 2002).

In most mammals and birds, sexual selection on male body size is *relatively* stronger than fecundity selection on female body size, resulting in male-biased SSD (Abouheif & Fairbairn,

1997). In invertebrates such as arthropods however, fecundity selection on female size generally exceeds sexual selection on male size, thus leading to female-biased SSD (Abouheif & Fairbairn, 1997; Blanckenhorn *et al.*, 2007a; Hirst & Kiorboe, 2014). Whether females or males are the larger sex has crucial implications for the mating system (eg. Ding & Blanckenhorn, 2002), but can also promote unequal variation in body size between sexes. Males tend to vary more strongly in size in taxa with male-biased SSD, whereas the opposite is observed in species where females are larger (Rensch's rule; Abouheif & Fairbairn, 1997).

The most important proximate mechanisms shaping biases in body size between the sexes and variation therein are differences in the juvenile period (so called sexual bimaturism: SBM) and differential rates of growth during this stage (Blanckenhorn *et al.*, 2007b; Teder, 2014); but see Budriene *et al.* (2013) for an example of SSD mediated by differences in size at hatching). Prolonged development as well as increased growth rates mediated by greater foraging effort can augment the risk of juvenile mortality, and because body size is playing a key role for adult reproductive success, selection on adult body size indirectly affects juvenile growth parameters (Teder, 2014). The relative importance of growth rate and development time is taxon specific, so the question of whether SSD is largely accounted for by sex-specific growth rates rather than development time differences remains undecided across a broad range of taxa (Blanckenhorn *et al.*, 2007b; but see Teder, 2014).

Variation in the strength and direction of SSD is usually strongest between species but also occurs among populations (eg. Teder & Tammaru, 2005; Young, 2005). Intraspecific variation in SSD is usually small and unidirectional. Thus far, only few studies find evidence for strong intraspecific directional variation in SSD, representing an ideal system to test the differential equilibrium model of the evolution of SSD (Badyaev & Hill, 2000; Puniamoorthy *et al.*, 2012b). Testing the model at the microevolutionary scale takes advantage of the relatively recent and potentially strong and ongoing evolutionary mechanisms that must have shaped the evident phenotypic divergence between populations. At the same time, the

shared evolutionary history of populations prevents highly divergent phylogenetic and developmental constraints, legitimating a direct comparison of populations.

A striking case of intraspecific variation in SSD was documented by Puniamoorthy *et al.* (2012b). SSD in the Holarctic black scavenger fly *Sepsis punctum* (Fabricius, 1794) was shown to be consistently reversed across continental populations: whereas males are the larger sex in Europe (EU), North American (NA) populations exclusively express female-biased SSD. The authors estimated univariate sexual selection differentials on male body size, fecundity selection differentials on female size, and (juvenile) viability selection in both sexes. Whereas fecundity and viability selection did not differ between populations, cross-continental variation in sexual selection on male size accounted for the directional reversal in SSD, leading to contrasting selective net effects between continents that support the differential equilibrium model of the evolution of SSD. As shown in other taxa, shifts in SSD are accompanied by changes in the mating system, often associated with elaborate changes in mating behavior (Blanckenhorn, 2005; Fairbairn, 2013; Cheng & Kuntner, 2014). In *S. punctum*, reversed SSD is accompanied by such a shift in mating system, evident in different re-mating frequencies and courtship display, likely re-enforcing variation in the strength of selection on male body size (Puniamoorthy *et al.*, 2012a).

The study of *S. punctum* nicely illustrates the strong effect of sexual selection on male body size on SSD. However, the association between SSD and sexual selection remains correlative and begs for replication in phylogenetically close, micro-evolutionary systems. I here document population differentiation in body size and SSD in another widespread, congeneric fly, *Sepsis neocynipsea* Melander and Spuler (1917), and demonstrate the convergent evolution of SSD reversals in the genus *Sepsis*. *S. neocynipsea* exhibits cross-continental directional variation in SSD, similar to *S. punctum*, but *vice versa*. To verify the same underlying selection principle in both species, I estimate sexual selection differentials on male size at three increasing operational sex ratios (OSRs) in *S. neocynipsea*, as well as the strength of fecundity selection on female size. Following the differential equilibrium model, cross-continental sex-specific variation in selection strength is expected.

I supplement this micro-evolutionary approach with comparative and phylogenetic analyses taking advantage of previously published body size data to set the evolution of SSD in sepsid flies into a phylogenetic perspective. I test whether sexual bimaturism (SBM) or sexual divergence in growth rates account for a higher proportion of variance in SSD and its direction and test for phylogenetic signals in body size and SSD.

## Materials & Methods

### Population sampling and fly culture maintenance

Sepsidae, or black scavenger flies, are a moderately species rich group of schizophoran flies (>300 species, 37 genera (Ozerov, 2005)) that are commonly found on cattle droppings or reproduce in various kinds of decaying organic matter (Pont & Meier, 2002; Rohner *et al.*, 2014). Due to the conspicuous male ornamentation and their suitability for laboratory experiments, sepsids are well suited for research in evolutionary genetics, behavioral ecology and ecotoxicology (Blanckenhorn, 1999; Ingram *et al.*, 2008; Puniamoorthy *et al.*, 2014). *S. neocynipsea*, the species studied here, is very common in North America, whereas its distribution in Europe is limited to the uplands of Central Europe and Great Britain (Pont & Meier, 2002; Rohner *et al.*, in press).

Wild-caught females were used to establish laboratory iso-female lines with several replicates per population for North America (Ramona, USA: 33.09°N,-116.79°E; Fort Hall, USA: 43.03°N,-112.44°E; Lamar Valley, USA: 44.6°N,-110.5°E; Meeker, USA: 40.04°N, -107.91°E; Lake Tahoe, USA: 39.1°N, -120°E; Kentucky, USA: 38.2°N, -84.87°E; Syracuse, USA: 42.94°N, -76.21°E) and Europe (Zurich, Switzerland: 47.4°N, 8.55°E; Sörenberg, Switzerland: 46.87°N, 8.27°E; Maggia, Switzerland: 46.25°N, 8.70°E; Oberwald, Switzerland: 46.53°N, 8.35°E; Fig. 1.1). Iso-female line culturing preserves natural genetic variation in the laboratory after capture but requires that the organism is not susceptible to inbreeding effects. All flies were provided with sugar, standardized cow dung and water *ad libitum*. The experimental procedure of Puniamoorthy *et al.* (2012b) was followed, with minor adaptations, to ensure comparability across the studies.


Figure 1: Sampling localities of the established iso-female lines of *S. neocynipsea* in North America and Europe. Note that *S. neocynipsea* is very rare in Europe, where its distribution is restricted to the uplands of Central Europe and Great Britain. Common garden experiments

To control for environmental variation in body size, offspring of the established iso-female lines were reared in a common environment. Following Puniamoorthy *et al.* (2012b), females were allowed to oviposit in previously frozen, standardized fresh cow dung for several hours, and the offspring were reared at low larval densities at constant 24°C, 60% humidity and 12 h light cycle. In addition to head width, a suitable estimate of overall body size (Blanckenhorn *et al.*, 2004), development time of all individuals was recorded. Under Rensch's rule, the relationship between male (y-axis) and female (x-axis) size or development time in log-log space should be characterized by a slope greater than 1 (Abouheif & Fairbairn, 1997).

## Estimating sexual selection on male size

Eggs of several iso-female lines per population were collected as for the common garden experiment, but larvae were reared under low and high densities, leading to variation in larval food competition and hence adult body size. Upon emergence, all eclosed flies were separated according to their sex and provided with water, sugar and cow dung. After four to five days (ensuring sexual maturity (Puniamoorthy *et al.*, 2012b)), mating trials under three increasing OSRs were conducted: five males and five females (OSR1), ten males and five females (OSR2), and twenty males and five females (OSR4). Mating trials were conducted in plastic containers equipped with sugar, water and dung, with 4-5 replicates per population and OSR. Copulating pairs were removed from the arena. After three to four hours, the trial was terminated and all individuals sacrificed by freezing. Subsequently, the head width of all males and females, irrespective of their mating success, was measured using a binocular microscope.

## Estimating fecundity selection on female size

To estimate fecundity selection, virgin females of varying sizes were paired with a male and housed in a glass vial equipped with sugar and dung for oviposition and incubated at 24°C. The presence of eggs was checked every day until the female laid her first clutch. The number of eggs, an estimate of female reproductive success, was then counted and measurements of the females' head width were taken.

## Statistical analysis of selection estimates

The common garden data (body size and development time) were analyzed using nested analysis of variance, with (iso-female) lines nested within populations nested within continents, and sex as a crossed factor.

Sexual selection differentials on male body size ($\beta_{SexS}$) were estimated using standard linear regression approaches (Arnold & Wade, 1984). For each mating trial replicate, the sizes of mated and unmated males were standardized by subtracting the replicate mean from each value and dividing by the standard deviation (i.e. *z*-scores). The resulting values approximate a normal distribution with a mean of zero and a standard deviation of one and

are thus comparable across populations, replicates and OSRs. Mating success of each male (0 or 1), estimating male reproductive success, was divided by the replicate mean mating success. When regressing relative mating success against standardized male body size, the slope of the regression line represents the standardized linear sexual selection differential ($\beta_{SexS}$). Replicates were added as random effect in the generalized linear model when calculating standard errors for $\beta_{SexS}$ for each OSR.

Selection differentials for female fecundity ($\beta_{FS}$) were calculated similarly. Female size was standardized by subtracting the population mean size and dividing by the standard deviation. Relative fecundity estimates were calculated by dividing each clutch size by the population mean.

To assess the significance of these selection coefficients, generalized linear (mixed) models were used with relative fitness (mating success or fecundity) as the outcome variable and standardized size as covariate (plus any other appropriate fixed factors). Interactions between standardized size and fixed factors indicate variation in selection.

## Assortative mating

Due to the low number of matings per replicate in the mating trials, the calculation of selection differentials for assortative mating by size would not yield robust estimates. I therefore did not calculate male fecundity selection differentials (by summing up sexual selection differentials and differentials reflecting the relative fitness gain due to assortative mating) as in Puniamoorthy *et al.* (2012b), but test for assortative mating on absolute, unstandardized body size estimates using a generalized linear model.

When investigating assortative mating by size, it is crucial to differentiate between "true" and "apparent" assortative mating (Arnqvist *et al.*, 1996). True assortative mating refers to a positive relationship between male and female size with a homogeneous distribution of residuals along the regression line. Apparent assortative mating on the other hand is caused by greater variation in mating success by either large or small males (i.e. when only small/large males are able to mate with females), resulting in an uneven distribution of residuals, i.e. a funnel-like pattern. FollowingArnqvist *et al.* (1996), the relationship between

the absolute residuals of the regression of male on female body size and absolute female size was investigated using Spearman's rank correlation coefficients (ϱ) for each continent separately, resulting in an estimate of the heteroscedasticity, an index of the type of assortative mating.

## Phylogenetic/comparative analyses

Sex-specific body size and development data for 23 sepsid taxa were extracted from Puniamoorthy *et al.* (2014), and complemented with common garden data for two continental populations of *Sepsis biflexuosa*, gathered using a common garden experiment. The most recent phylogenetic hypothesis for the Sepsidae proposed by Zhao *et al.* (2013) was used to reconstruct sex-specific body size and dimorphism of ancestral nodes applying Wagner's (linear) parsimony in Mesquite v3.03 (Maddison & Maddison, 2008) while setting branch lengths to one (the ancestral node value representing the median of both daughter and the parent node values). To test for phylogenetic clustering, terminal nodes were randomized 1000 times to estimate the frequency distribution of the parsimony score under a null model. If phenotypes are distributed randomly across the phylogeny, the parsimony score of the observed tree should fall within the 2.5$^{th}$ and 97.5$^{th}$ percentiles of the null model.

To identify the proportion of shared variance between body size, development time and growth rate differences between the sexes, squared correlation coefficients ($R^2$) were used. Indices for sexual bimaturism (SBM) and sexual growth rate difference were calculated following Blanckenhorn *et al.* (2007b) based on absolute development times (in days) and growth rates (= body size/development time).

$$SBM\ and\ sexual\ growth\ rate\ difference = \left( 100 * \frac{male\ trait\ value - female\ trait\ value}{male\ trait\ value + female\ trait\ value / 2} \right)$$

For SSD, an index (SDI) proposed by Lovich and Gibbons (1992) was used, arbitrarily defined as negative if males are larger than females, which has better statistical properties than simple ratios (Fairbairn, 2007).

$$SDI = \frac{size\ of\ larger\ sex}{size\ of\ smaller\ sex} - 1$$

In addition to an ordinary least squares regression, Felsenstein's independent contrasts (Felsenstein, 1985) and a phylogenetic generalized linear model (PGLS; Freckleton *et al.*, 2002) were performed to account for the phylogenetic non-independence between species.

All analyses were conducted in either SPSS v22 (IBM, 2013) or R v3.1.3 (R Development Core Team, 2008).

# Results

## Common garden experiments

SSD is clearly reversed between continents in *S. neocynipsea*, with European populations having female-biased SSD whereas males are the larger sex in North American populations (continent by sex interaction: $F_{1,8} = 93.33$, $P < 0.001$; Fig. 2; Table 1; Appendix: Table A1). SSD varies significantly between lines (sex by line interaction: $F_{49,963} = 1.64$, $P = 0.004$) but not among populations within continents (sex by population interaction: $F_{8,49} = 1.08$, $P = 0.392$). In contrast to *S. punctum*, overall body size does not differ between continents ($F_{1,8} = 0.83$, $P = 0.78$). However, there is population differentiation in body size (population nested within continent: $F_{8,50} = 10.34$, $P < 0.001$).

*S. neocynipsea* also shows intraspecific sex-dependent variation among continents in development time (sex by continent interaction: $F_{1,8} = 44.40$, $P = 0.002$, Fig. 2; Appendix: Table A2). North American males (12.68 ± 0.05 (SE) days) take longer to develop than females (11.94 ± 0.06 days) at 24°C, whereas the sexes do not differ significantly in development time in Europe (females: 12.86 ± 0.06 days; males: 12.76 ± 0.07 days).

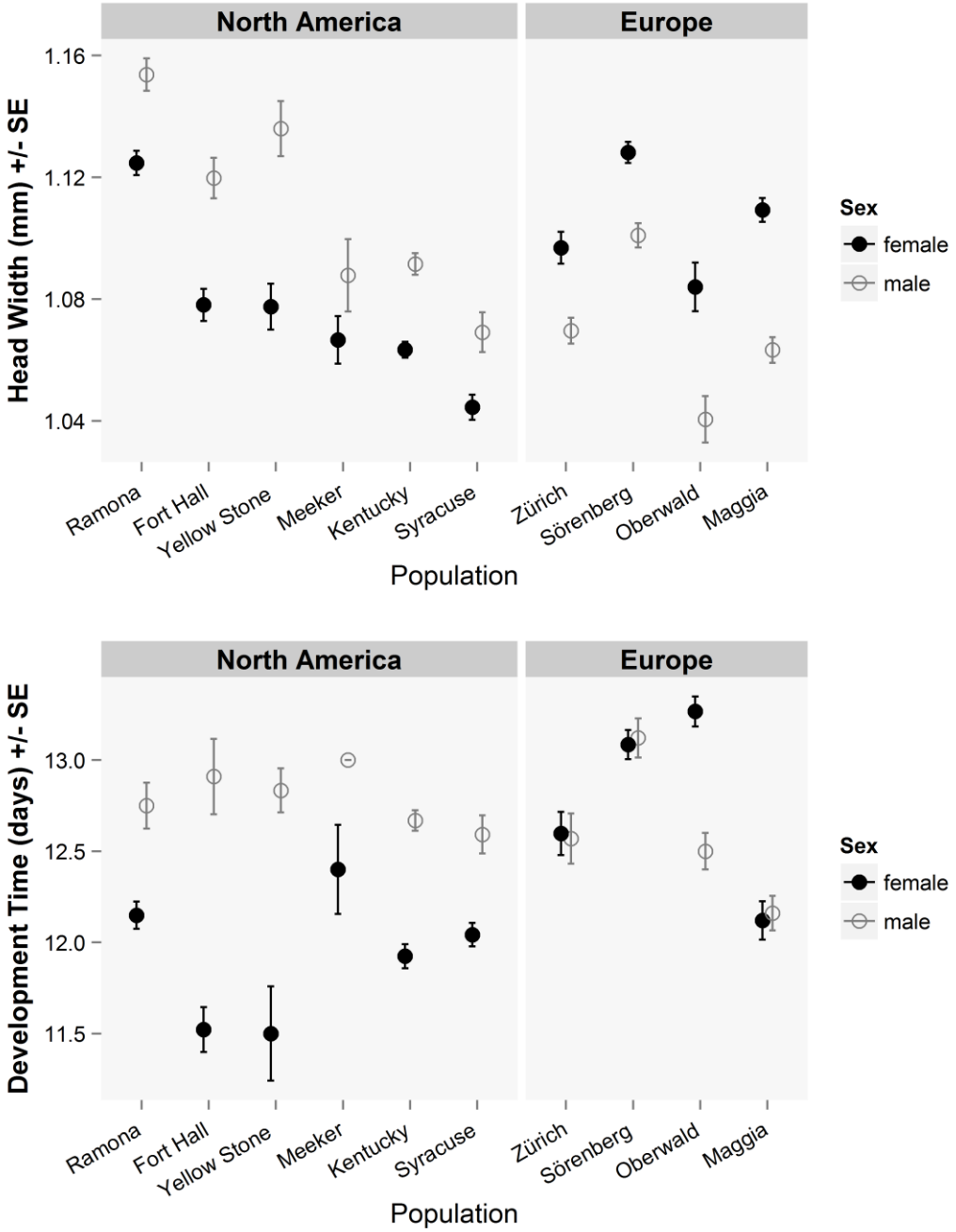


Figure 2: Cross-continental reversal of sexual size dimorphism in *Sepsis neocynipsea* at 24°C in a laboratory common garden (top). While males are the larger sex in North American populations, SSD is female-biased in European populations. In North America females have a shorter development than males, whereas development time does not differ between the sexes in three of the four European populations (bottom), nor overall.

Table 1: Head width (mm) and development time (days) for North American and European populations of *S. neocynipsea*.

| Population | | | Head Width (mm) | | Development Time (days) | |
|---|---|---|---|---|---|---|
| **Europe** | **Sex** | **N** | **Mean** | **SD** | **Mean** | **SD** |
| Maggia | female | 25 | 1.11 | 0.02 | 12.12 | 0.53 |
| | male | 25 | 1.06 | 0.02 | 12.16 | 0.47 |
| Oberwald | female | 30 | 1.08 | 0.04 | 13.27 | 0.45 |
| | male | 26 | 1.04 | 0.04 | 12.50 | 0.51 |
| Sörenberg | female | 107 | 1.13 | 0.04 | 13.08 | 0.83 |
| | male | 91 | 1.10 | 0.04 | 13.12 | 1.02 |
| Zürich | female | 67 | 1.10 | 0.04 | 12.60 | 0.97 |
| | male | 58 | 1.07 | 0.03 | 12.57 | 1.04 |
| **North America** | | | | | | |
| Fort Hall | female | 23 | 1.08 | 0.03 | 11.52 | 0.59 |
| | male | 22 | 1.12 | 0.03 | 12.91 | 0.97 |
| Kentucky | female | 158 | 1.06 | 0.03 | 11.92 | 0.83 |
| | male | 172 | 1.09 | 0.05 | 12.67 | 0.73 |
| Meeker | female | 5 | 1.07 | 0.02 | 12.40 | 0.55 |
| | male | 5 | 1.09 | 0.03 | 13.00 | 0.00 |
| Ramona | female | 49 | 1.13 | 0.03 | 11.78 | 1.90 |
| | male | 64 | 1.15 | 0.04 | 12.75 | 1.01 |
| Syracuse | female | 71 | 1.04 | 0.03 | 12.04 | 0.55 |
| | male | 49 | 1.07 | 0.05 | 12.59 | 0.73 |
| Yellowstone | female | 18 | 1.08 | 0.03 | 11.50 | 1.10 |
| | male | 18 | 1.14 | 0.04 | 12.83 | 0.51 |

Plotting the natural logarithm of the mean size of each sex per line against each other shows a positive relationship for body size between the sexes, indicating a genetic correlation between the sexes (Fig.3). There are differences in male size between the continents (continent main effect: $F_{1,8} = 27.69$, $P = 0.001$; generalized linear model using populations as random effect), but no significant difference in the slope of the regression between continents (female size by continent interaction: $F_{1,47} = 0.66$, $P = 0.421$). Since on either continent the slopes do not differ from 1 (main diagonal), these data do not support Rensch's rule at the intraspecific level (slope of regression line with standard error: EU: 0.85 ± 0.12; NA: 0.96 ± 0.16).

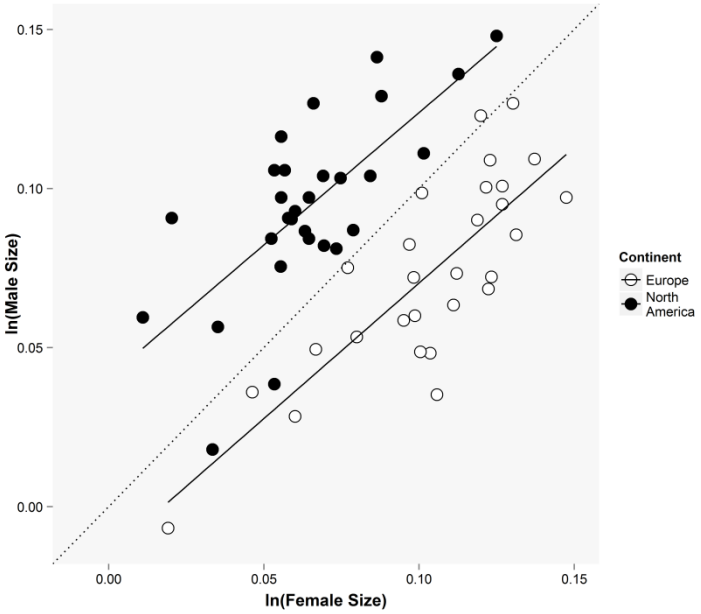


Figure 3: Major axis regression of male on female body size per iso-female line in log-log space. The hatched line indicates the isometric slope. Black dots refer to North American, gray dots to European *S. neocynipsea* lines.

## Estimating sexual and fecundity selection

Sexual selection differentials are overall positive (North America: 35/41; Europe: 20/30), thus favoring large male size, and tend to increase with OSR in North America as predicted by sexual selection theory (Emlen & Oring, 1977), while remaining constant in Europe (Fig. 4). There is, however, strong variation between and within populations (Table 2).

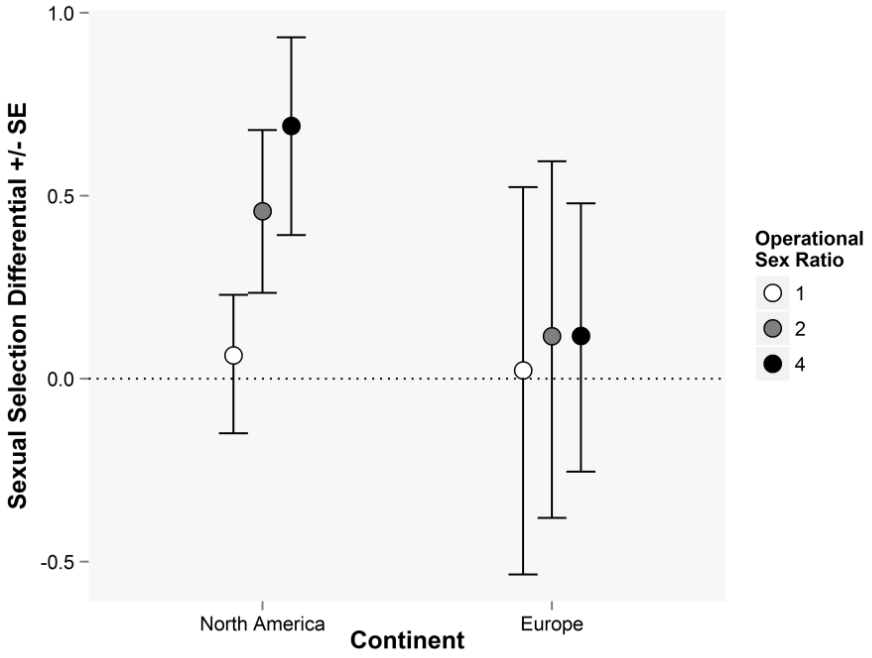


Figure 4: Linear sexual selection differentials on male body size (±1SE) for North American and European populations of *S. neocynipsea*. Shading indicates the increasing operational sex ratios (OSR1: 5 females and 5 females; OSR2: 5 females and 10 males; OSR4: 5 females and 20 males).

Overall, body size has a significant effect on male mating success ($X^2(1) = 14.4$, $P < 0.001$; Appendix: Table A3) and its intensity varies between continents (Continent by size interaction: $X^2(1) = 10.3$, $P = 0.001$) and populations (Populations nested within continent by size interaction: $X^2(6) = 14.1$, $P = 0.029$), as well with OSR (OSR by size interaction: $X^2(2) = 7.6$, $P = 0.022$). The proportion of females that copulated within each replicate was added as covariate to control for variation in female receptivity (which is highly dependent on the female's egg laying cycle; Teuschl & Blanckenhorn, 2007).

Fecundity selection differentials are all positive, varying between 0.03 and 0.31 (Table 1). Consequently, larger females are more fecund ($X^2(1) = 28.46$, $P < 0.000$; Appendix: Table A4) when applying a generalized linear model. However there is no significant difference in the effect of size between continents or populations (size by continent interaction: $X^2(1) = 0.51$, $P = 0.477$; size by population nested within continent: $X^2(5) = 6.96$, $P = 0.224$).

Table 2: Female fecundity selection ($\beta_{FS}$) and male sexual selection differentials ($\beta_{SexS}$) for all populations and OSRs.

| Population | Female clutch size | Male mating success | |
|---|---|---|---|
| **Europe** | **$\beta_{FS}$ ±SE** | **OSR** | **$\beta_{SexS}$ ±SE** |
| Sörenberg | 0.280 ± 0.140 | 1 | 0.417 ± 0.322 |
| | | 2 | 0.764 ± 0.372 |
| | | 4 | 0.234 ± 0.348 |
| Maggia | 0.080 ± 0.040 | 1 | -0.214 ± 0.310 |
| | | 2 | -0.908 ± 0.538 |
| | | 4 | 0.249 ± 0.356 |
| Oberwald | 0.028 ± 0.014 | 1 | 0.124 ± 0.369 |
| | | 2 | -0.267 ± 0.343 |
| | | 4 | -0.131 ± 0.349 |
| Zürich | 0.312 ± 0.156 | 1 | -0.284 ± 0.350 |
| | | 2 | 0.545 ± 0.432 |
| | | 4 | 0.321 ± 0.421 |
| **North America** | | | |
| Kentucky | 0.247 ± 0.123 | 1 | 0.038 ± 0.268 |
| | | 2 | 0.731 ± 0.228 |
| | | 4 | 0.952 ± 0.227 |
| Ramona | NA | 1 | -0.059 ± 0.293 |
| | | 2 | 0.634 ± 0.274 |
| | | 4 | -0.013 ± 0.357 |
| Syracuse | 0.189 ± 0.094 | 1 | 0.298 ± 0.232 |
| | | 2 | 0.315 ± 0.226 |
| | | 4 | 0.938 ± 0.227 |
| Yellowstone | 0.127 ± 0.063 | 1 | -0.053 ± 0.331 |
| | | 2 | 0.115 ± 0.221 |
| | | 4 | 0.723 ± 0.314 |

## Assortative mating

Large females mate with large males (male size as covariate: $X^2(1) = 10.96$, $P = 0.001$; Appendix: Table A5), indicating positive assortative mating by size which is typical in animals (Jiang *et al.*, 2013), the strength of assortment however varies between continents (continent by male size interaction: $X^2(1) = 10.99$, $P = 0.001$). Residuals are homogeneously distributed along the regression line, suggesting true rather than apparent assortative mating (North America: $\varrho = -0.01$, $P = 0.883$; Europe: $\varrho = -0.02$, $P = 0.838$; Fig.5).

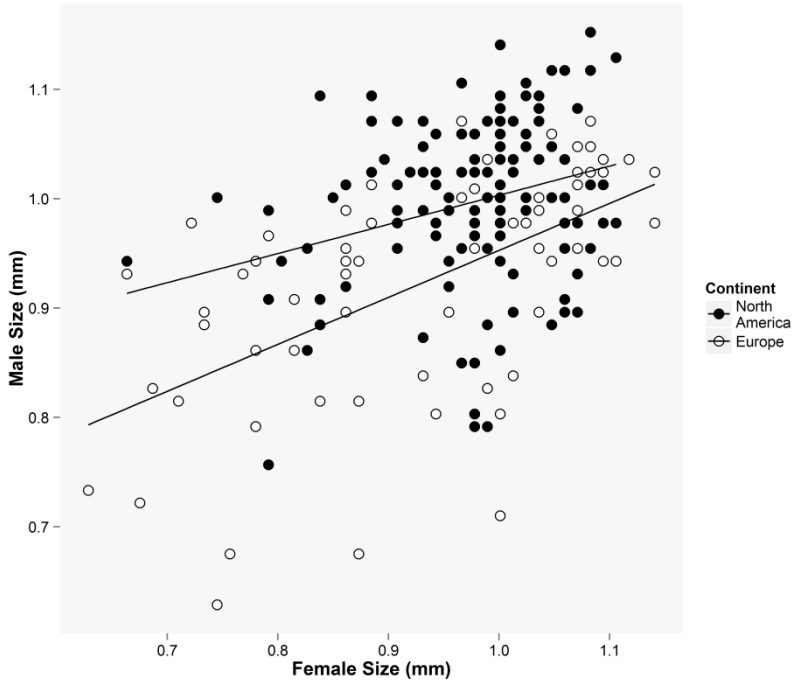


Figure 5: *Sepsis neocynipsea* shows weak positive assortative mating, the strength of which varies between continents. Residuals are distributed evenly.

## Phylogenetic/comparative analyses

Ancestral state reconstruction using Wagner's parsimony revealed the dynamic evolution of body size in both sexes with several instances of body size increases and decreases (Fig. 6). Female-biased SSD is most common within the Sepsidae; however, Wagner's parsimony suggests that male-biased SSD (here SDI < 0) evolved at least four times independently within the genus *Sepsis*: in *S. neocynipsea* (North American populations), *S. punctum* (European populations), *S. thoracica* and *S. lateralis;* Fig. 7*).* Randomization tests revealed a phylogenetic signal for male ($P = 0.009$) and female body size ($P = 0.006$), but not for SDI ($P = 0.289$).

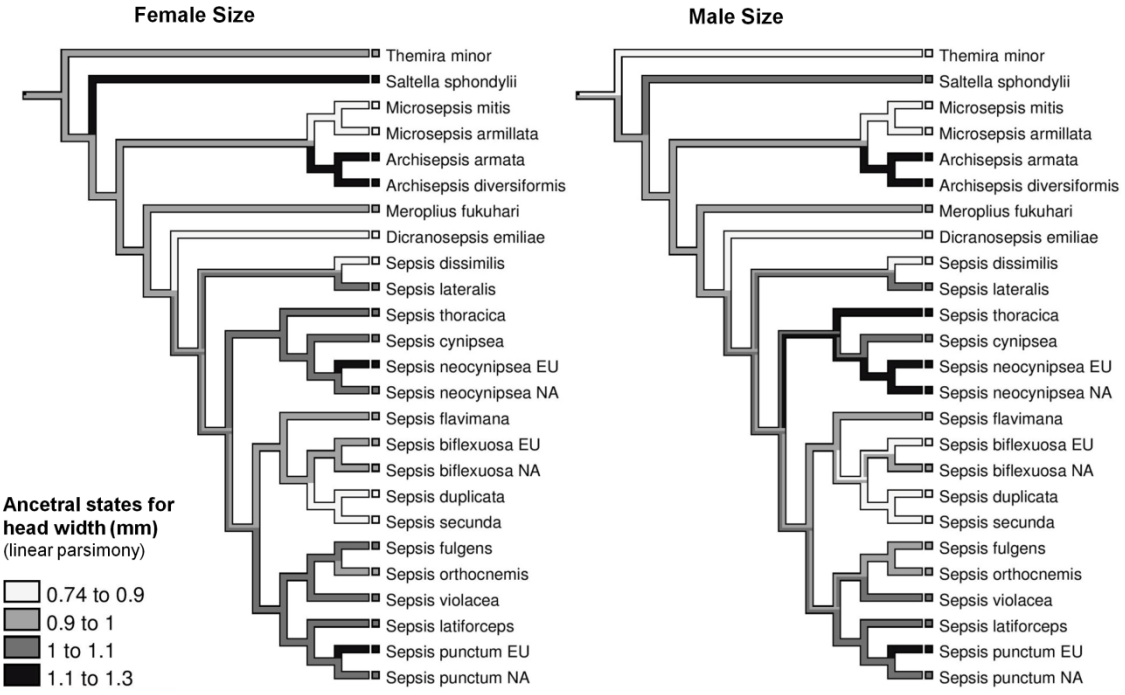


Figure 6: The estimated phylogenetic history of body size is dynamic in both sexes. Randomization tests reveal phylogenetic signals for male and female body size.

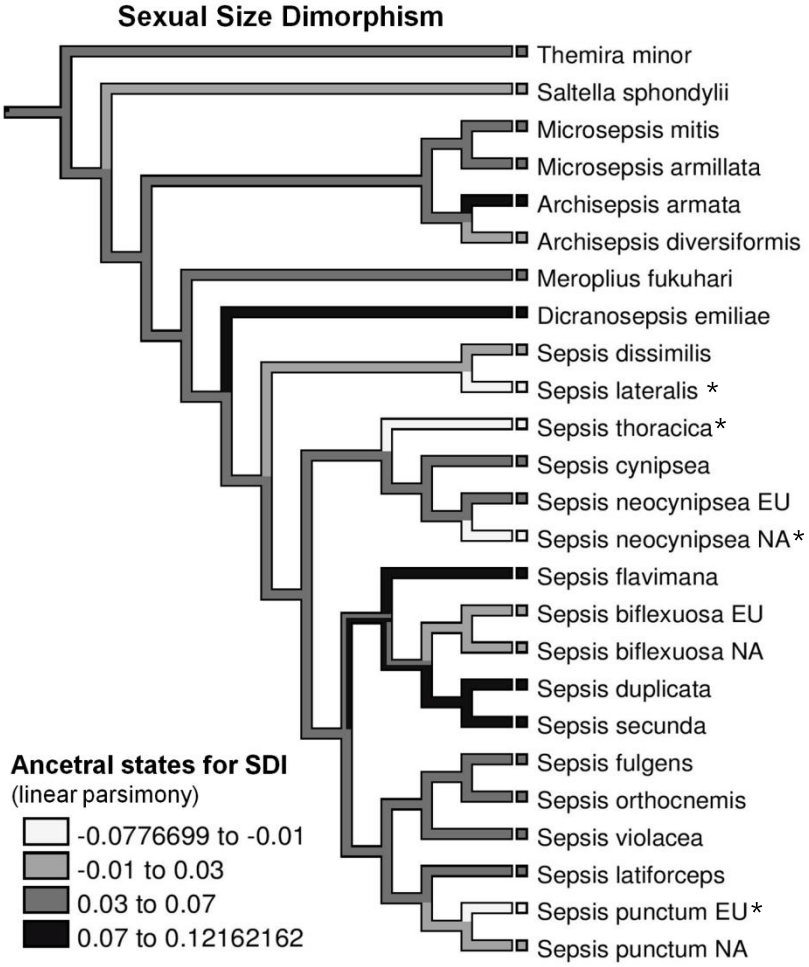


Figure 7: Linear parsimony reveals multiple origins of reversals in SSD. Male biased SSD (here SDI < 0, indicated with an asterisk) evolved at least four times convergently, while female-biased SSD is by far the most common pattern in the species covered here (SDI > 0). Thus, SSD evolves independently and rapidly in the Sepsidae.

Comparative analyses revealed that a higher proportion of variance in body size (mean between the sexes) and SSD is explained by growth rate than by variation in development time (Fig. 8). This pattern remains when applying Felsenstein's independent contrasts (Fig. 9) or PGLS (Table 3).

Table 3: Consistently more variance in body size (top) and SSD (bottom) is explained by growth rate rather than development time, irrespective of which model is used (Pagel's λ is a branch length transformation factor and indicates the strength of the phylogenetic signal accounted for in the model, see Munkemuller *et al.*, 2012).

| **Method** | **Body Size ~ Growth Rate** | | **Body Size ~ Development Time** | |
|---|---|---|---|---|
| | $R^2$ | P | $R^2$ | P |
| Ordinary least squares regression (λ = 0) | 0.406 | < 0.001*** | 0.043 | 0.322 |
| Independent contrasts (λ = 1) | 0.203 | 0.027* | 0.019 | 0.521 |
| PGLS (0 < λ < 1) | 0.183 | 0.033* | 0.032 | 0.395 |

| **Method** | **SDI ~ Growth Rate Difference** | | **SDI ~ Sexual Bimaturism** | |
|---|---|---|---|---|
| | $R^2$ | P | $R^2$ | P |
| Ordinary least squares regression (λ = 0) | 0.799 | < 0.001*** | 0.681 | < 0.001*** |
| Independent contrasts (λ = 1) | 0.739 | < 0.001*** | 0.641 | < 0.001*** |
| PGLS (0 < λ < 1) | 0.804 | < 0.001*** | 0.706 | < 0.001*** |

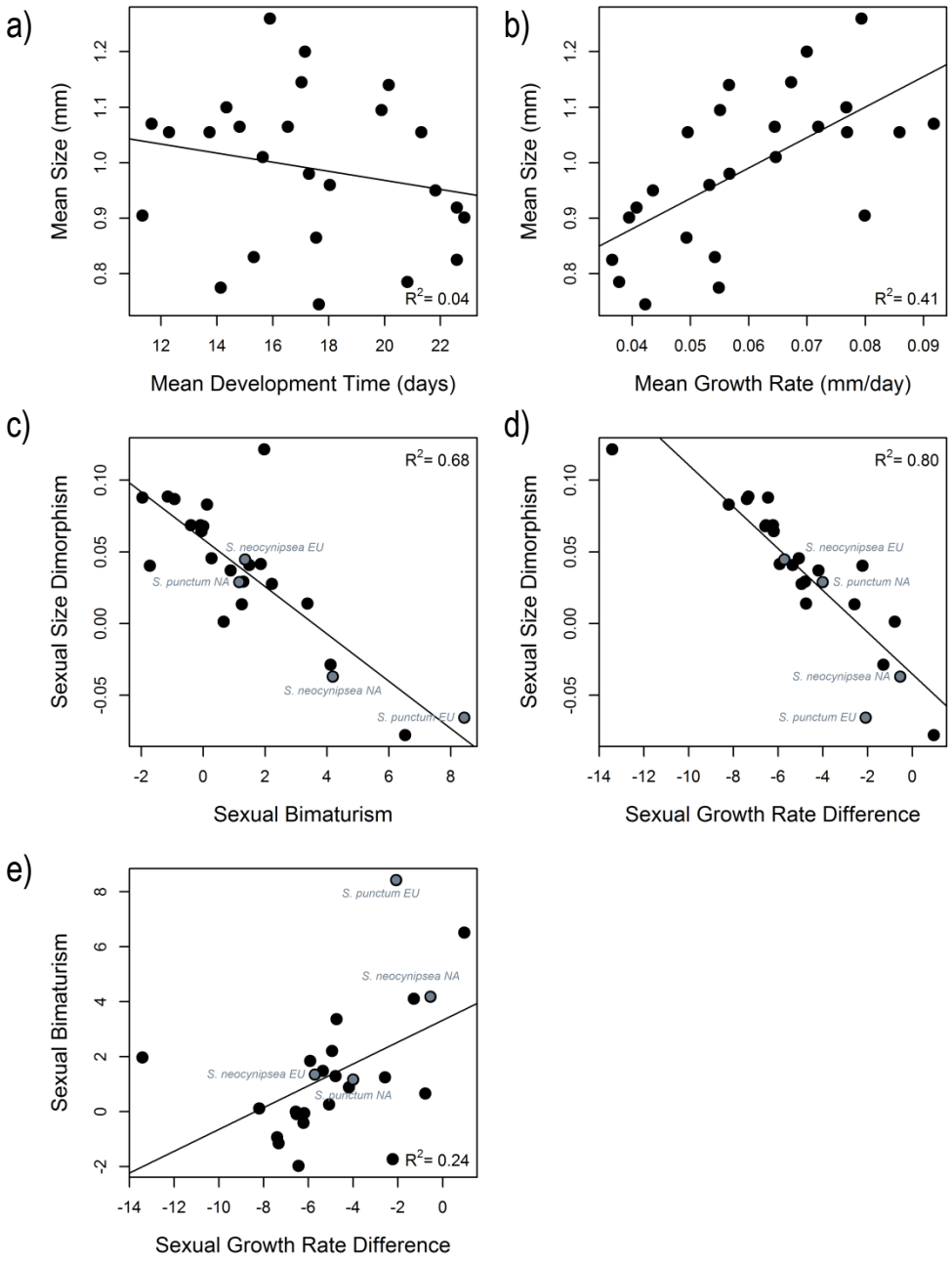


Figure 8: Relationships between species specific mean body size (head width), development time and growth rates (a,b). Analogously, indices of SBM and sexual difference in growth rates are plotted against SDI (c,d,e). $R^2$ indicates the fit of the least squared regression.

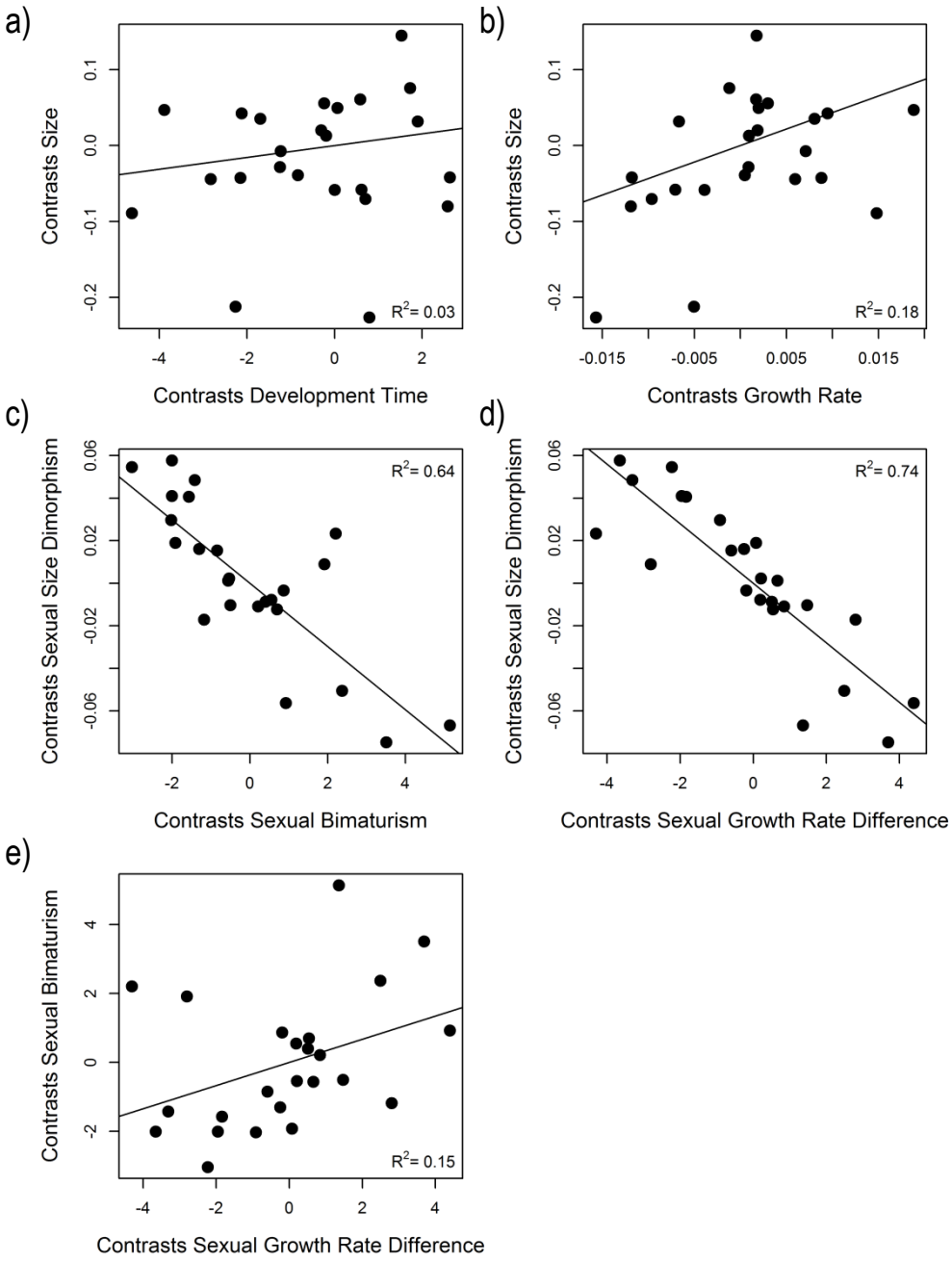


Figure 9: Relationships between phylogenetically independent contrasts for species specific mean size, development time and growth rate (a,b). Concordantly, contrasts for indices of SBM and sexual difference in growth rates are plotted against SDI (all indices are based on non-transformed size measures; c,d,e). $R^2$ indicates the fit of the least squared regression (forced through origin).

# Discussion

I here document a cross-continental reversal of SSD in *Sepsis neocynipsea*, with European populations having female-biased SSD and North American populations male-biased SSD, while lacking support for Rensch's rule in the intraspecific level. Male-biased SSD is clearly associated with an increase in the strength of sexual selection favoring large male body size in North America relative to Europe, whereas fecundity selection on female size does not vary between the continents. Therefore, *S. neocynipsea* represents the second species of *Sepsis* that has undergone shifts in the direction of SSD, likely as a response to intensified sexual selection on male size. These findings again support the differential equilibrium model of the evolution of SSD (Fairbairn *et al.*, 2007). Further comparative analyses suggest that the extent and direction of SSD evolved independently and rapidly within the genus *Sepsis*, mostly mediated by sexual divergence in growth rates. In the following, I discuss these findings, their limitations and their implications for our current understanding of the evolution of SSD.

## Limitations of the differential equilibrium model

Theory predicts that the net effects of sex-specific selection on body size should qualitatively and *quantitatively* predict the degree of SSD, provided no genetic or ontogenetic constrains hamper sexual divergence (Andersson, 1994; Blanckenhorn, 2007). Estimating reliable selection differentials is difficult, especially in the field, and requires high sample sizes, replication and phenotypic variation amongst populations. Most importantly, the fitness response measured should be a good correlate of lifetime reproductive success.

I here investigated two central fitness components: male mating success and female fecundity in the laboratory. Due to the artificial and simplified nature of laboratory experiments, estimates of these selection components do not necessarily correlate strongly with life-time reproductive success in the field (Clutton-Brock, 1988; Hunt *et al.*, 2004). However, male mating success and early female fecundity doubtlessly contribute to overall fitness in relatively short lived species and should be target of strong selection (Andersson,

1994; Shuker & Simmons, 2014). Nevertheless, other traits also contribute to overall fitness, particularly viability and other naturally selected traits. I did not estimate viability selection, or any other fitness components, for several reasons. (1) In contrast to female and male reproductive success, adult viability under laboratory conditions and especially intrinsic (residual) life expectancy may not be under direct selection in the field (analogous to the wall of death; Hamilton, 1966). (2) Especially in holometabolous insects, each life stage is characterized by its own, very distinct ecology and is thus potentially subjected to contrasting selective pressures especially on body size (Andersson, 1994). Reliable estimates of viability selection differentials thus need to be estimated in the field and should additionally include immature stages, which in the case of such a small species is unfeasible (Blanckenhorn, 2007). In fact, continental variation in viability selection on body size in the field can certainly be expected due to presumably different ecological niches of North American and European *S. neocynipsea* populations: this species inhabits high-altitude environments in the Swiss Alps (and is rarely if ever found anywhere else; Pont & Meier, 2002; Rohner *et al.*, in press), but is far more common in North America, also at low elevations, including desert environments in Arizona, suggesting continent-specific thermal adaptation and selection on associated physiological traits, including size. These effects however are not expected to vary between the sexes.

## The mating system of *S. neocynipsea*

The mating system of any species is not simply a consequence of adaptations in response to sexual and other selection, but also in turn imposes variation in selective forces itself, leading to reciprocal feedbacks (Kokko *et al.*, 2014). Male size is under sexual selection in *S. neocynipsea*, the intensity of which varies between continents, so the mating systems are expected to co-vary. Although mating systems seem to be hard-wired in the short term (Ding & Blanckenhorn, 2002), and should be robust to small variations in SSD caused by sexual selection, evolution of a directional reversal should induce (or be caused by) variation in the mating system (eg. in reproductive behavior or the degree of polyandry). The direction of

SSD indeed plays a major role in attraction and intrasexual competition, but also in the ability to control the outcome of mating (Ding & Blanckenhorn, 2002). In general, species with female biased SSD tend to show (indirect or direct) female choice and scramble competition polygyny (small male advantage; Emlen & Oring, 1977; Andersson, 1994; Blanckenhorn *et al.*, 1995). On the other hand, systems with male biased SSD often feature male resource defense polygyny, territoriality and in some cases forced copulations (Blanckenhorn, 2005; Fairbairn, 2013; Shuker & Simmons, 2014).

*S. neocynipsea* typically mate at their oviposition or feeding substrate (as do *Sepsis spp.* in general), where numerous males await the arrival of a receptive female (Eberhard, 1999; Pont & Meier, 2002). Copulations are characterized by at times complex (pre-) copulatory courtship including tactile, sometimes visual stimulation of the female (Puniamoorthy *et al.*, 2009) or the transfer of chemical compounds (the evolutionary significance of which is still unknown; see Araujo *et al.*, 2014). Males of most species, including *S. neocynipsea*, jump onto the female and grab her wing bases with their strongly modified fore femora to ride on her back, trying to establish genital contact while displaying a broad array of courtship behavior (Puniamoorthy *et al.*, 2009). Aggressive takeovers by other males can occur in *S. neocynipsea* (Rohner, pers. observ.), as have been described in the common European sister species *S. cynipsea* (Parker, 1972), and females that are reluctant to mate can usually prevent copulation by bending their abdomen downward and by vigorously shaking off their unwanted mates (which could also act as quality assessment; Blanckenhorn *et al.*, 2000; Hunt & Sakaluk, 2014). In contrast to *S. punctum*, I could not detect any obvious systematic differences in mating behavior between European and North American *S. neocynipsea*, the observed differences in assortment by size could however point to a certain degree of mating system divergence.

Sexual selection for large male size could be mediated by several mechanisms in *S. neocynipsea*. If the male is larger than its mate, he may have better opportunities to control the act of mating and coerce copulations, though this is rare. Larger males could also be more successful in intrasexual competition. Eberhard (1999) describes courtship behavior of a North American population of *S. neocynipsea* at a female feeding site as follows: "[…] males of *S. neocynipsea* did not defend consistently delimited territories, but rather attempted to

mount or drive off other males in their immediately vicinity as they moved about", anecdotally suggesting some sort of male-male competition for access to females. But also forms of mate choice could be responsible for positive selection on male size: I found evidence for *true* rather than *apparent* assortative mating (a weak and positive relationship), implying that individuals copulate with mates of similar size and that despite strong sexual selection on male size, large males do not mate randomly, i.e. with females of all sizes, but rather with females of similar size, suggesting direct or indirect forms of mate choice (Arnqvist *et al.*, 1996; Rowe & Arnqvist, 1996). There are numerous adaptive and non-adaptive hypotheses aiming to explain assortative mating (reviewed in Jiang *et al.*, 2013), including physical or mechanic constraints for mating and indirect effects of the mating system. The mechanisms involved in the pattern observed in the Sepsidae remains unknown, but assortment could be mediated via by-products of intra- and intersexual competition (Jiang *et al.*, 2013, and references therein), i.e. female preference for large males and intrasexual competition among males for access to large and more fecund females.

In this context it is worth noting that all (sub)taxa with male-biased SSD (*S. thoracica, S. lateralis, S. neocynipsea NA, S. punctum EU*) also show a much brighter, sometimes even yellowish color (Rohner, pers. observ), which is in sharp contrast to the common appearance of black scavenger flies (*Sepsis spp.*). Whether coloration is under selection (eg. bright colors as costly display) or is a physiological side effect of large males in these species is currently under investigation.

## Multiple independent origins of reversed SSD

Until a few decades ago, evolutionary ecologists considered SSD a clearly defined and invariant trait of species and higher taxa. More recent studies however depict moderate and sometimes strong SSD variation within and between taxa in response to environmental conditions but also selective regimes (Pearson *et al.*, 2002; Teder & Tammaru, 2005; Fairbairn *et al.*, 2007; Hudson & Fu, 2013), although directional reversals of SSD within taxa are very rare. Parsimony suggests that reversals in SSD occurred at least four times independently

within the genus *Sepsis*, twice even at the intraspecific level. Furthermore, male-biased SSD evolved independently in cross-continental populations of *S. neocynipsea* and *S. punctum*, yet at the same time conspecific populations cannot be discerned with confidence based on morphology in either species. Thus, SSD reversals seem to evolve relatively fast in this clade, as quickly as behavioral and sexually selected morphological traits. In addition, in contrast to male and female size, SSD does not show a phylogenetic signal in sepsid flies, again suggesting independent and relatively labile evolutionary trajectories. Therefore, sex-specific alterations of growth control and physiology appear to be rather unconstrained in this group.

Larval growth of flies is complex and strictly controlled by various endocrine cascades (Colombani *et al.*, 2005; Testa *et al.*, 2013; Mirth *et al.*, 2014; Nijhout *et al.*, 2014). Larvae neither grow continuously nor in a linear fashion; therefore estimates of linear growth rates (as used here) might be misleading and drastically underestimate the actual speed of growth (Blanckenhorn, 1999; Tammaru *et al.*, 2010; Testa *et al.*, 2013). Unfortunately, more sophisticated and realistic growth estimates are currently lacking and would require detailed growth trajectories for each species separately (Teder, 2014).

Growth rate and development time are traded-off at the larval stages while determining reproductive success in the adult stage. Especially in *Sepsis* species that generally develop in cow dung, a patchy resource characterized by high inter- and intraspecific competition for food and space (Hanski & Cambefort, 1991; Emlen, 2014), fast growth should be weighted against extending the growth period (i.e. development time) in determining adult body size. Blanckenhorn *et al.* (2007b) suggested that (linearized) growth rates, rather than developmental durations, seem to be responsible for more of the variation in SSD across a broad range of taxa, and especially within the Sepsidae. However, Teder (2014), focusing on insects also, reached the opposite conclusion for most taxa. The two studies greatly differ in their taxon sampling, especially the relative number of species of moths. Especially in Lepidoptera, however, protandry itself might be under sexual selection (eg. Wiklund & Solbreck, 1982; Thornhill & Alcock, 1983), and thus these two studies might contradict each other simply due to taxon sampling. In addition, several insect taxa modify their

development time via alternative mechanisms, eg. by adding instars rather than extending instar duration, which seems frequent in Orthoptera (eg. Berner & Blanckenhorn, 2006; Kelly & Adams, 2010) and Lepidoptera (Kingsolver, 2007), potentially limiting ontogenetic homology and comparability across taxa because adding an instar might provoke different selective pressures and ontogenetic constraints than extension of instars.

For the sepsids studied here, sexual divergence in growth rates induced more variation in SSD and body size than sexual bimaturism in all analyses applied (ordinary least squares, Felsenstein's independent contrasts and PGLS), supporting the pattern originally suggested by Blanckenhorn *et al.* (2007b). In our case of cross-continental directional reversals of SSD in *S. neocynipsea* and especially *S. punctum* however, both sexual divergence in growth rates and bimaturity seem to proximately mediate variation in SSD. In conclusion, the relative importance of development times vs. growth rates probably highly depends on the taxon investigated, their ecology and ontogeny, and therefore, a general trend might remain elusive or may not exist at all.

### Are cross-continental populations of *S. neocynipsea* and *S. punctum* conspecific?

One could argue that population differentiation in SSD between continents in both *S. neocynipsea* and *S. punctum* is strong, and that the associated variation in the mating system (at least in *S. punctum*: Puniamoorthy *et al.*, 2012a,b) could support the need for elevating cross-continental populations of both species to the (sub)species level. Yet, allopatric populations cannot be identified by genital or foreleg morphology, which are classic characters used in the taxonomy of this group (Pont & Meier, 2002; Rohner *et al.*, 2014 ; Fig. A1&2). Also, cross-continental populations of both species produce viable hybrid offspring under laboratory conditions (F1 and F2; Puniamoorthy, Giesen & Rohner, pers. observ.), and COI differences are moderate between the continental populations (COI distance for *S. punctum* ≅ 3% (Puniamoorthy *et al.*, 2012b); estimates for *S. neocynipsea* are currently lacking, but the distance between *S. neocynipsea* and its sister species *S. cynipsea* amount to only < 1.34% (Puniamoorthy *et al.*, 2009), thus, intraspecific variation is expected to be even lower).

The COI gene may not be involved in speciation (Kwong *et al.*, 2012) but points to a certain degree of phylogenetic independency of cross-continental populations. Therefore, different species concepts yield different conclusions. Concepts based on reproductive isolation (Mayr, 2000) and morphology will refute the species-level for continental populations in both species, whereas phylogenetic species concepts (Wiley & Mayden, 2000) weight their independent phylogenetic fates more, however, monophyly cannot be assessed with the currently available genetic data. I contend that both *S. neocynipsea* and *S. punctum* are in the process of undergoing incipient allopatric speciation, and that the phylogenetic independence between allopatric populations should be taken into account. However, I doubt that the declaration of a (sub)species level in either species makes sense in light of the current understanding of their genetic, morphological and behavioral divergence.

## Conclusions

I here demonstrate that *Sepsis neocynipsea* features cross-continental variation in the direction of SSD, likely mediated by variation in the strength of sexual selection on male body size. Furthermore, the direction of SSD (and probably the associated shifts in the mating system) in the Sepsidae are far more variable than previously presumed, as reversed SSD evolved multiple times convergently, hinting towards labile and independent evolutionary trajectories of sex-specific larval growth parameters. Focusing on the complex growth of juvenile flies (Mirth & Riddiford, 2007; Tammaru *et al.*, 2010; Testa *et al.*, 2013; Nijhout *et al.*, 2014), future research should address the proximate causes of these rapid shifts in size dimorphism in the Sepsidae and the resolution of underlying constraints, such as (inter)sexual (onto)genetic conflict or genetic correlations between the sexes (Badyaev, 2002).

## Acknowledgements

I would like to thank all the people who supported me during my MSc-studies. First and foremost, I thank Prof. Wolf U. Blanckenhorn and Dr. Nalini Puniamoorthy for their excellent and inspiring guidance and supervision. Special thanks go to Martin A. Schäfer and the rest of the Blanckenhorn research group for the fruit- and joyful time, and to all my friends and family for their great support.

# References

Abouheif, E. & Fairbairn, D.J. (1997) A comparative analysis of allometry for sexual size dimorphism: Assessing Rensch's rule. *American Naturalist*, **149**, 540-562.

Andersson, B. (1994) *Sexual selection*. Princeton University Press.

Araujo, D.P., Tuan, M.J.M., Yew, J.Y. & Meier, R. (2014) Analysing small insect glands with uv-ldi ms: High-resolution spatial analysis reveals the chemical composition and use of the osmeterium secretion in *Themira superba* (Sepsidae: Diptera). *Journal of Evolutionary Biology*, **27**, 1744-1750.

Arnold, S.J. & Wade, M.J. (1984) On the measurement of natural and sexual selection: Theory. *Evolution*, **38**, 709-719.

Arnqvist, G., Rowe, L., Krupa, J.J. & Sih, A. (1996) Assortative mating by size: A meta-analysis of mating patterns in water striders. *Evolutionary Ecology*, **10**, 265-284.

Badyaev, A.V. (2002) Growing apart: An ontogenetic perspective on the evolution of sexual size dimorphism. *Trends in Ecology & Evolution*, **17**, 369-378.

Badyaev, A.V. & Hill, G.E. (2000) The evolution of sexual dimorphism in the house finch. I. Population divergence in morphological covariance structure. *Evolution*, **54**, 1784-1794.

Bateman, A.J. (1948) Intra-sexual selection in *Drosophila*. *Heredity*, **2**, 349-368.

Berner, D. & Blanckenhorn, W.U. (2006) Grasshopper ontogeny in relation to time constraints: Adaptive divergence and stasis. *Journal of Animal Ecology*, **75**, 130-139.

Blanckenhorn, W.U. (1999) Different growth responses to temperature and resource limitation in three fly species with similar life histories. *Evolutionary Ecology*, **13**, 395-409.

Blanckenhorn, W.U. (2000) The evolution of body size: What keeps organisms small? *Quarterly Review of Biology*, **75**, 385-407.

Blanckenhorn, W.U. (2005) Behavioral causes and consequences of sexual size dimorphism. *Ethology*, **111**, 977-1016.

Blanckenhorn, W.U. (2007) Case studies of the differential-equilibrium hypothesis of sexual size dimorphism in two dung fly species. *Sex, size, and gender roles: Evolutionary studies of sexual size dimorphism* (ed. by D.J. Fairbairn, W.U. Blanckenhorn and T. Szekely), pp. 106-114. Oxford Univ. Press, London, UK.

Blanckenhorn, W.U., Preziosi, R.F. & Fairbairn, D.J. (1995) Time and energy constraints and the evolution of sexual size dimorphism - to eat or to mate. *Evolutionary Ecology*, **9**, 369-381.

Blanckenhorn, W.U., Meier, R. & Teder, T. (2007a) Rensch's rule in insects: Patterns among and within species. *Sex, size, and gender roles: Evolutionary studies of sexual size dimorphism* (ed. by D.J. Fairbairn, W.U. Blanckenhorn and T. Szekely), pp. 60-70. Oxford Univ. Press, London, UK.

Blanckenhorn, W.U., Kraushaar, U.R.S., Teuschl, Y. & Reim, C. (2004) Sexual selection on morphological and physiological traits and fluctuating asymmetry in the black scavenger fly *Sepsis cynipsea*. *Journal of Evolutionary Biology*, **17**, 629-641.

Blanckenhorn, W.U., Muhlhauser, C., Morf, C., Reusch, T. & Reuter, M. (2000) Female choice, female reluctance to mate and sexual selection on body size in the dung fly *Sepsis cynipsea*. *Ethology*, **106**, 577-593.

Blanckenhorn, W.U., Dixon, A.F.G., Fairbairn, D.J., Foellmer, M.W., Gibert, P., van der Linde, K., Meier, R., Nylin, S., Pitnick, S., Schoff, C., Signorelli, M., Teder, T. & Wiklund, C. (2007b) Proximate causes of rensch's rule: Does sexual size dimorphism in arthropods result from sex differences in development time? *American Naturalist*, **169**, 245-257.

Budriene, A., Budrys, E. & Nevronyte, Z. (2013) Sexual size dimorphism in the ontogeny of the solitary predatory wasp *Symmorphus allobrogus* (Hymenoptera: Vespidae). *Comptes Rendus Biologies*, **336**, 57-64.

Cheng, R.C. & Kuntner, M. (2014) Phylogeny suggests nondirectional and isometric evolution of sexual size dimorphism in argiopine spiders. *Evolution*, **68**, 2861-2872.

Clutton-Brock, T.H. (1988) *Reproductive success: Studies of individual variation in contrasting breeding systems*. University of Chicago Press.

Colombani, J., Bianchini, L., Layalle, S., Pondeville, E., Dauphin-Villemant, C., Antoniewski, C., Carre, C., Noselli, S. & Leopold, P. (2005) Antagonistic actions of ecdysone and insulins determine final size in *Drosophila*. *Science*, **310**, 667-670.

Darwin, C. (1872) *The descent of man, and selection in relation to sex*. D. Appleton.

Ding, A. & Blanckenhorn, W.U. (2002) The effect of sexual size dimorphism on mating behaviour in two dung flies with contrasting dimorphism. *Evolutionary Ecology Research*, **4**, 259-273.

Eberhard, W.G. (1999) Mating systems of sepsid flies and sexual behavior away from oviposition sites by *Sepsis neocynipsea* (Diptera : Sepsidae). *Journal of the Kansas Entomological Society*, **72**, 129-130.

Emlen, D.J. (2014) Reproductive contests and the evolution of extreme weaponry. *The evolution of insect mating systems* (ed. by D. Shuker and L. Simmons). OUP Oxford.

Emlen, S.T. & Oring, L.W. (1977) Ecology, sexual selection, and evolution of mating systems. *Science*, **197**, 215-223.

Fairbairn, D.J. (2007) Introduction: The enigma of sexual size dimorphism. *Sex, size, and gender roles: Evolutionary studies of sexual size dimorphism* (ed. by D.J. Fairbairn, W.U. Blanckenhorn and T. Szekely), pp. 1-10. Oxford Univ. Press, London, UK.

Fairbairn, D.J. (2013) *Odd couples: Extraordinary differences between the sexes in the animal kingdom*. Princeton University Press.

Fairbairn, D.J., Blanckenhorn, W.U. & Székely, T. (2007) *Sex, size and gender roles: Evolutionary studies of sexual size dimorphism*. OUP Oxford.

Felsenstein, J. (1985) Phylogenies and the comparative method. *American Naturalist*, **125**, 1-15.

Freckleton, R.P., Harvey, P.H. & Pagel, M. (2002) Phylogenetic analysis and comparative data: A test and review of evidence. *American Naturalist*, **160**, 712-726.

Hamilton, W.D. (1966) Moulding of senescence by natural selection. *Journal of Theoretical Biology*, **12**, 12-&.

Hanski, I. & Cambefort, Y. (1991) *Dung beetle ecology*. Princeton University Press, Princeton.

Hedrick, A.V. & Temeles, E.J. (1989) The evolution of sexual dimorphism in animals - hypotheses and tests. *Trends in Ecology & Evolution*, **4**, 136-138.

Hirst, A.G. & Kiorboe, T. (2014) Macroevolutionary patterns of sexual size dimorphism in copepods. *Proceedings of the Royal Society B-Biological Sciences*, **281**

Hudson, C.M. & Fu, J.Z. (2013) Male-biased sexual size dimorphism, resource defense polygyny, and multiple paternity in the Emei moustache toad (*Leptobrachium boringii*). *Plos One*, **8**

Hunt, J. & Sakaluk, S.K. (2014) Mate choice. *The evolution of insect mating systems* (ed. by D. Shuker and L. Simmons), pp. 129-158. OUP Oxford.

Hunt, J., Bussiere, L.F., Jennions, M.D. & Brooks, R. (2004) What is genetic quality? *Trends in Ecology & Evolution*, **19**, 329-333.

IBM, C. (2013) *IBM SPSS statistics for windows,*. IBM Corp.

Ingram, K.K., Laamanen, T., Puniamoorthy, N. & Meier, R. (2008) Lack of morphological coevolution between male forelegs and female wings in *Themira* (Sepsidae: Diptera: Insecta). *Biological Journal of the Linnean Society*, **93**, 227-238.

Jiang, Y.X., Bolnick, D.I. & Kirkpatrick, M. (2013) Assortative mating in animals. *American Naturalist*, **181**, E125-E138.

Kelly, C.D. & Adams, D.C. (2010) Sexual selection, ontogenetic acceleration, and hypermorphosis generates male trimorphism in Wellington Tree weta. *Evolutionary Biology*, **37**, 200-209.

Kingsolver, J.G. (2007) Variation in growth and instar number in field and laboratory *Manduca sexta*. *Proceedings of the Royal Society B-Biological Sciences*, **274**, 977-981.

Kokko, H., Klug, H. & Jennions, M.D. (2014) Mating systems. *The evolution of insect mating systems* (ed. by D. Shuker and L. Simmons). OUP Oxford.

Kwong, S., Srivathsan, A., Vaidya, G. & Meier, R. (2012) Is the COI barcoding gene involved in speciation through intergenomic conflict? *Molecular Phylogenetics and Evolution*, **62**, 1009-1012.

Lovich, J.E. & Gibbons, J.W. (1992) A review of techniques for quantifying sexual size dimorphism. *Growth Development and Aging*, **56**, 269-281.

Maddison, W.P. & Maddison, D.R. (2008) Mesquite: A modular system for evolutionary analysis. **Version 3.03**

Mayr, E. (2000) The biological species concept. *Species concepts and phylogenetic theory: A debate* (ed. by Q.D. Wheeler and R. Meier), pp. 17-29. Columbia University Press, New York.

Melander, A.L. & Spuler, A. (1917) *The dipterous families sepsidae and piophilidae*. State College of Washington, Agricultural Experiment Station.

Mirth, C.K. & Riddiford, L.M. (2007) Size assessment and growth control: How adult size is determined in insects. *Bioessays*, **29**, 344-355.

Mirth, C.K., Tang, H.Y., Makohon-Moore, S.C., Salhadar, S., Gokhale, R.H., Warner, R.D., Koyama, T., Riddiford, L.M. & Shingleton, A.W. (2014) Juvenile hormone regulates body size and perturbs insulin signaling in *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of America*, **111**, 7018-7023.

Munkemuller, T., Lavergne, S., Bzeznik, B., Dray, S., Jombart, T., Schiffers, K. & Thuiller, W. (2012) How to measure and test phylogenetic signal. *Methods in Ecology and Evolution*, **3**, 743-756.

Nijhout, H.F., Riddiford, L.M., Mirth, C., Shingleton, A.W., Suzuki, Y. & Callier, V. (2014) The developmental control of size in insects. *Wiley Interdisciplinary Reviews-Developmental Biology*, **3**, 113-134.

Ozerov, A.L. (2005) World catalogue of the family Sepsidae (insecta: Diptera). *Zoologicheskie issledovania (Zoological Studies)*, **8**, 1–74.

Parker, G.A. (1972) Reproductive behavior of *Sepsis cynipsea* (Diptera-Sepsidae) .1. Preliminary analysis of reproductive strategy and its associated behavior patterns. *Behaviour*, **41**, 172-&.

Pearson, D., Shine, R. & Williams, A. (2002) Geographic variation in sexual size dimorphism within a single snake species (*Morelia spilota*, Pythonidae). *Oecologia*, **131**, 418-426.

Peters, R.H. (1986) *The ecological implications of body size*. Cambridge University Press.

Pont, A.C. & Meier, R. (2002) The Sepsidae (Diptera) of Europe. *Fauna Entomologica Scandinavica*, **37**, 1-221.

Preziosi, R.F. & Fairbairn, D.J. (2000) Lifetime selection on adult body size and components of body size in a waterstrider: Opposing selection and maintenance of sexual size dimorphism. *Evolution*, **54**, 558-566.

Price, T.D. (1984) The evolution of sexual size dimorphism in darwin finches. *American Naturalist*, **123**, 500-518.

Puniamoorthy, N., Blanckenhorn, W.U. & Schafer, M.A. (2012a) Differential investment in pre- vs. post-copulatory sexual selection reinforces a cross-continental reversal of sexual size dimorphism in *Sepsis punctum* (Diptera: Sepsidae). *Journal of Evolutionary Biology*, **25**, 2253-2263.

Puniamoorthy, N., Schafer, M.A. & Blanckenhorn, W.U. (2012b) Sexual selection accounts for the geographic reversal of sexual size dimorphism in the dung fly, *Sepsis punctum* (Diptera: Sepsidae). *Evolution*, **66**, 2117-2126.

Puniamoorthy, N., Ismail, M.R.B., Tan, D.S.H. & Meier, R. (2009) From kissing to belly stridulation: Comparative analysis reveals surprising diversity, rapid evolution, and much homoplasy in the mating behaviour of 27 species of sepsid flies (Diptera: Sepsidae). *Journal of Evolutionary Biology*, **22**, 2146-2156.

Puniamoorthy, N., Schäfer, M.A., Römbke, J., Meier, R. & Blanckenhorn, W.U. (2014) Ivermectin sensitivity is an ancient trait affecting all ecdysozua but shows phylogenetic clustering among sepsid flies. *Evolutionary Applications*, **7**, 548-554.

RDevelopmentCoreTeam (2008) *R: A language and environment for statistical computing*.

Reiss, M.J. (1991) *The allometry of growth and reproduction*. Cambridge University Press.

Rohner, P.T., Ang, Y., Lei, Z., Puniamoorthy, N., Blanckenhorn, W.U. & Meier, R. (2014) Genetic data confirm the species status of *Sepsis nigripes* Meigen (Diptera: Sepsidae) and adds one species to the alpine fauna while questioning the synonymy of *Sepsis helvetica* Munari. *Invertebrate Systematics*, **28**, 555-563.

Rohner, P.T., Bächli, G., Pollini, L., Jochmann, R., Duelli, P., Obrist, M.K. & Blanckenhorn, W.U. (in press) Distribution, diversity gradients and rapoport's elevational rule in the black scavenger flies of the swiss alps (Diptera: Sepsidae). *Insect Conservation and Diversity*,

Rowe, L. & Arnqvist, G. (1996) Analysis of the causal components of assortative mating in water striders. *Behavioral Ecology and Sociobiology*, **38**, 279-286.

Shuker, D. & Simmons, L. (2014) *The evolution of insect mating systems*. OUP Oxford.

Tammaru, T., Esperk, T., Ivanov, V. & Teder, T. (2010) Proximate sources of sexual size dimorphism in insects: Locating constraints on larval growth schedules. *Evolutionary Ecology*, **24**, 161-175.

Teder, T. (2014) Sexual size dimorphism requires a corresponding sex difference in development time: A meta-analysis in insects. *Functional Ecology*, **28**, 479-486.

Teder, T. & Tammaru, T. (2005) Sexual size dimorphism within species increases with body size in insects. *Oikos*, **108**, 321-334.

Testa, N.D., Ghosh, S.M. & Shingleton, A.W. (2013) Sex-specific weight loss mediates sexual size dimorphism in *Drosophila melanogaster*. *Plos One*, **8**

Teuschl, Y. & Blanckenhorn, W.U. (2007) The reluctant fly: What makes *Sepsis cynipsea* females willing to copulate? *Animal Behaviour*, **73**, 85-97.

Thornhill, R. & Alcock, J. (1983) *The evolution of insect mating systems*. Harvard University Press.

Wiklund, C. & Solbreck, C. (1982) Adaptive versus incidental explanations for the occurrence of protandry in butterfly, *Leptidea sinapis* L. *Evolution*, **36**, 56-62.

Wiley, E.O. & Mayden, R.L. (2000) The evolutionary species concept. *Species concepts and phylogenetic theory: A debate* (ed. by Q.D. Wheeler and R. Meier), pp. 70-89. Columbia University Press, New York.

Young, K.A. (2005) Life-history variation and allometry for sexual size dimorphism in pacific salmon and trout. *Proceedings of the Royal Society B-Biological Sciences*, **272**, 167-172.

Zhao, L., Annie, A.S., Amrita, S., Yi, S.K. & Rudolf, M. (2013) Does better taxon sampling help? A new phylogenetic hypothesis for Sepsidae (Diptera: Cyclorrhapha) based on 50 new taxa and the same old mitochondrial and nuclear markers. *Molecular Phylogenetics and Evolution*, **69**, 153-64.

# Appendix

Table A1: SPSS output: ANOVA summary table for head width (mm).

**Dependent variable: Head width (mm)**

| source | | sums of squares (type III) | df | Mean sums of squares | F | Sig. |
|---|---|---|---|---|---|---|
| intercept | Hypothesis | 667.483 | 1 | 667.483 | 12054.399 | .000 |
| | Error | .437 | 7.891 | .055[a] | | |
| Continent | Hypothesis | .006 | 1 | .006 | .083 | .780 |
| | Error | . 568 | 8 | .071 | | |
| Population(Continent) | Hypothesis | .568 | 8 | .071 | 10.341 | .000 |
| | Error | .343 | 50 | .007 | | |
| Line(Population(Continent)) | Hypothesis | .343 | 50 | .007 | 3.810 | .000 |
| | Error | .090 | 49.890 | .002[d] | | |
| Sex | Hypothesis | .000 | 1 | .000 | .123 | .733 |
| | Error | .017 | 9.933 | .002[e] | | |
| Sex * Continent | Hypothesis | .183 | 1 | .183 | 93.335 | .000 |
| | Error | .016 | 8 | .002 | | |
| Sex * Population(Continent) | Hypothesis | .016 | 8 | .002 | 1.081 | .392 |
| | Error | .089 | 49 | .002 | | |
| Sex * | Hypothesis | .089 | 49 | .002 | 1.644 | .004 |
| Line(Population(Continent)) | Error | 1.061 | 963 | .001[h] | | |

Table A.2: SPSS output: ANOVA summary table for development time (days).

**Dependent variable: Development time (days)**

| source | | sums of squares (type III) | df | Mean sums of squares | F | Sig. |
|---|---|---|---|---|---|---|
| Intercept | Hypothesis | 77182.769 | 1 | 77182.769 | 13535.454 | .000 |
| | Error | 35.925 | 6.300 | 5.702[a] | | |
| Continent | Hypothesis | 11.730 | 1 | 11.730 | 1.557 | .259 |
| | Error | 45.216 | 6 | 7.536 | | |
| Population(Continent) | Hypothesis | 45.216 | 6 | 7.536 | 1.323 | .268 |
| | Error | 244.958 | 43 | 5.697 | | |
| Line(Population(Continent)) | Hypothesis | 244.958 | 43 | 5.697 | 11.705 | .000 |
| | Error | 21.320 | 43.806 | .487[d] | | |
| Sex | Hypothesis | 5.806 | 1 | 5.806 | 3.525 | .101 |
| | Error | 12.049 | 7.316 | 1.647[e] | | |
| Sex * Continent | Hypothesis | 24.253 | 1 | 24.253 | 11.896 | .014 |
| | Error | 12.232 | 6 | 2.039 | | |
| Sex * Population (Continent) | Hypothesis | 12.232 | 6 | 2.039 | 4.203 | .002 |
| | Error | 20.371 | 42 | .485 | | |
| Sex * | Hypothesis | 20.371 | 42 | .485 | .836 | .762 |
| Line( Population(Continent)) | Error | 522.359 | 900 | .580[h] | | |

Table A3: SPSS output: Generalized linear model with male mating success as binomial response variable.

**Dependent variable: Male mating success (binomial; [0,1])**

| | Type III | | |
|---|---|---|---|
| source | Wald-Chi-square | df | Sig. |
| Intercept | **90.211** | **1** | **.000** |
| Continent | **1.059** | **1** | **.303** |
| Population(Continent) | **1.381** | **6** | **.967** |
| OSR | **.996** | **2** | **.608** |
| Standardized size (z-score) | **14.384** | **1** | **.000** |
| Mating probability | **41.957** | **1** | **.000** |
| Continent * Standardized size | **10.338** | **1** | **.001** |
| Population(Continent) * Standardized size | **14.074** | **6** | **.029** |
| OSR * Standardized size | **7.606** | **2** | **.022** |

Table A4: SPSS output: Generalized linear model with female reproductive success as response variable.

**Dependent variable: Clutch size (number of eggs)**

| | Type III | | |
|---|---|---|---|
| source | Wald-Chi-square | df | Sig. |
| Continent | **.335** | **1** | **.563** |
| Population(Continent) | **6.315** | **5** | **.277** |
| Tibia size | **28.459** | **1** | **.000** |
| Continent * tibia size | **.506** | **1** | **.477** |
| Population(Continent) * tibia size | **6.957** | **5** | **.224** |

Table A4: SPSS output: Generalized linear model with female reproductive success as response variable.

**Dependent variable: Female size (head width in mm)**

| source | Typ III Wald-Chi-square | df | Sig. |
|---|---|---|---|
| Intercept | .105 | 1 | .746 |
| Continent | 11.661 | 1 | .001 |
| Population(Continent) | 15.895 | 6 | .014 |
| Replicate(Population(Continent)) | 33.390 | 23 | .075 |
| male head width | 10.958 | 1 | .001 |
| OSR | 1.230 | 2 | .541 |
| Continent * male head width | 10.993 | 1 | .001 |
| Population(Continent) * male head width | 13.999 | 6 | .030 |
| Replicate(Population(Continent)) * male head width | 32.147 | 23 | .097 |
| Continent * OSR | 2.401 | 2 | .301 |
| OSR * male head width | 1.578 | 2 | .454 |
| Continent * OSR * male head width | 2.615 | 2 | .270 |
| Population(Continent) * OSR * male head width | 41.328 | 12 | .000 |

Figure A1: Typical foreleg morphology of *Sepsis neocynipsea* for females and males of both continental populations. Note that even though some disparity in male foreleg morphology exists between continental populations, the great variation within populations prohibits unambiguous identification of populations.

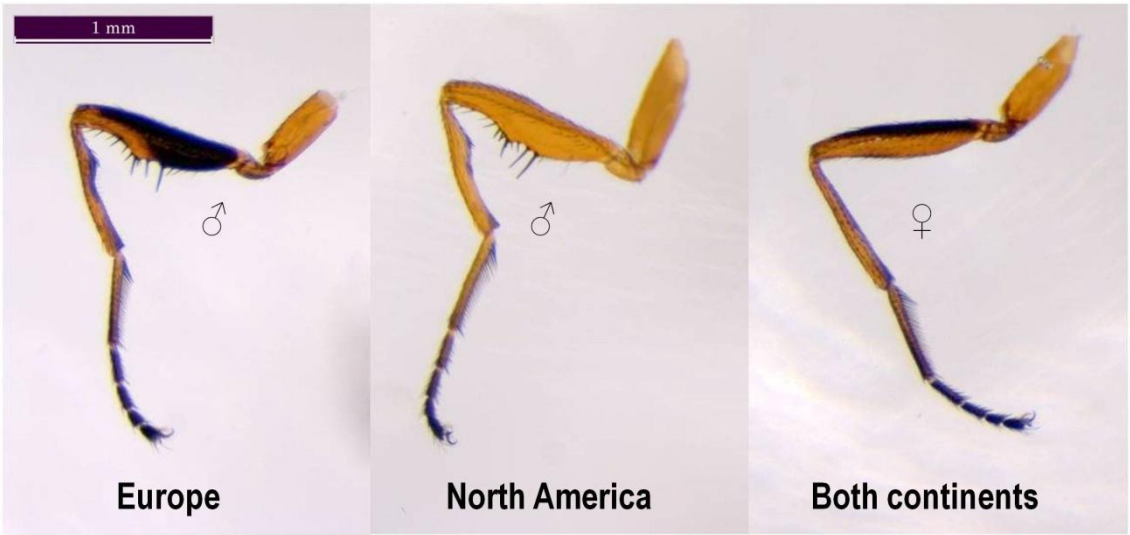

Figure A2: Typical habitus of North American and European *Sepsis neocynipsea*.

## Statement of Authorship:

I declare that I have used no other sources and aids other than those indicated. All passages quoted from publications or paraphrased from these sources are indicated as such, i.e. cited and/or attributed. This thesis was not submitted in any form for another degree or diploma at any university or other institution of tertiary education.

Zurich, 03.07.2015

Patrick T. Rohner

# Curriculum vitae

## Personal information

| | |
|---|---|
| Date of birth | 28.02.1992 |
| Nationality | Swiss |

## Education

| | | |
|---|---|---|
| **BSc in Biology** | University of Zurich | 2011-2014 |
| **MSc in Systematics and Evolution** | University of Zurich<br>Institute of Evolutionary Biology and Environmental Studies | 2014-2015 |

## University appointments

**Laboratory assistant** with Prof. Wolf Blanckenhorn at the since 2012
Institute of Evolutionary Biology and Environmental Sciences
University of Zurich

**Laboratory technician** with Prof. Stephan Neuhauss at the since 2013
Institute of Molecular Life Sciences
University of Zurich

## Awards & Grants

**Semester prize** of the University of Zurich for the project: 2012
«Some Like It Small, Some Like It Big: Sexual Size Dimorphism in *Sepsis neocynipsea* and other Sepsid Flies »

**Forschungskredit** of the University of Zurich 2015
Full financial support covering 19 months in the frame work of a PhD in Evolutionary Biology.

## Publications and submitted manuscripts

**Rohner** P. T., Ang Y., Zhao L., Puniamoorthy N., Blanckenhorn W.U., Meier R. (2014) *Genetic data confirm the species status of Sepsis nigripes Meigen, 1826 (Diptera: Sepsidae) and adds one species to the Alpine fauna while questioning the synonymy of Sepsis helvetica Munari, 1985.* Invertebrate Systematics 28, 555–563.

Ang Y., **Rohner** P. T., Meier R. (2015) Across the Baltic: a new record for an enigmatic black scavenger fly, *Zuskamira inexpectata* (Pont, 1987) (Sepsidae) in Finland. Biodiversity Data Journal 3: e4308. doi: 10.3897/BDJ.3.e4308.

**Rohner** P. T., Bächli G., Pollini L., Duelli P., Obrist M., Jochmann R., Blanckenhorn W. U. (2015) *Distribution, diversity gradients and Rapoport's elevational rule in the black scavenger flies of the Swiss Alps (Diptera: Sepsidae).* Insect Conservation and Diversity. (in press)

Blanckenhorn W. U., **Rohner**, P. T., Bernasconi, M. V., Haugstetter, J. and Buser, A. *Is quantitative mass barcoding of dung fauna biodiversity feasible?* Submitted to Environmental Toxicology and Chemistry.

## Presentations at national and international meetings

**Rohner** P.T., Ang Y., Zhao L., Puniamoorthy N., Blanckenhorn W.U., Meier R. (2014) *Morphology vs. molecules – species boundaries in two rare dung fly species (Diptera; Sepsidae*). Swiss Systematics Society Meeting 2014 in Geneva, Switzerland. **oral**

**Rohner** P. T., Blanckenhorn W.U. and Puniamoorthy N. (2014) *Cross-continental variation in sexual selection and its effect on the contrasting reversal of sexual size dimorphism in closely related sepsid fly species*. 8th International Congress of Dipterology, Potsdam, Germany. **oral**

**Rohner** P. T. and Blanckenhorn W.U. (2014) *A shitload of flies: Surprisingly rich alpine fauna of dung flies in Switzerland (Diptera: Sepsidae)*. 8th International Congress of Dipterology, Potsdam, Germany. **poster**

**Rohner** P. T., Ang Y., Zhao L., Puniamoorthy N., Blanckenhorn W.U. and Meier R. (2014) *Genetic data confirm the species status of Sepsis nigripes Meigen, 1826 (Diptera: Sepsidae) and adds one species to the Alpine fauna while questioning the synonymy of Sepsis helvetica Munari, 1985.* 8th International Congress of Dipterology, Potsdam, Germany. **poster**

**Rohner** P. T., Blanckenhorn W.U. and Puniamoorthy N. (2014) *Cross-continental variation in sexual selection: Contrasting reversal of sexual size dimorphism in closely related sepsid flies*. biology14 conference, Geneva, Switzerland. **poster**

**Rohner** P. T. and Blanckenhorn W. U. (2013) *Kryptische Sepsiden der Schweiz*. Meeting of the Entomological Society of Zurich, Zurich, Switzerland. **oral**

**Rohner** P. T., Blanckenhorn W.U. and Puniamoorthy N. (2013) *Cross-continental variation in sexual selection: Contrasting reversal of sexual size dimorphism in closely related sepsid flies*. 14th Congress of the European Society for Evolutionary Biology, Lisbon, Portugal. **poster**

## Reviewer functions

*Insect Conservation and Diversity*

*Physiological Entomology*

*Cladistics*

*Ecology and Evolution*

## Professional memberships

European Society for Evolutionary Biology

Swiss Systematics Society

Swiss Entomological Society

Entomological Society of Zurich